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# Comparison of the Intramuscular, Intranasal or Sublingual Routes of Midazolam Administration for the Control of Soman-Induced Seizures\*

John H. McDonough, Kerry E. Van Shura, John C. LaMont, Joseph D. McMonagle and Tsung-Ming Shih

Pharmacology Branch, Research Division, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, USA

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**Abstract:** This study evaluated the anticonvulsant effectiveness of midazolam to stop seizures elicited by the nerve agent soman when midazolam was administered by different routes (intramuscular, intranasal or sublingual) at one of two different times after the onset of seizure activity. Guinea pigs previously prepared with cortical electrodes to record brain electroencephalographic activity were pre-treated with pyridostigmine (0.026 mg/kg, intramuscularly) 30 min. before challenge with a seizure-inducing dose of the nerve agent soman (56 µg/kg, subcutaneously), and 1 min. later, they were administered 2.0 mg/kg atropine sulfate admixed with 25.0 mg/kg 2-PAM Cl (intramuscularly). Groups of animals were administered differing doses of midazolam by the intramuscular, intranasal or sublingual route at either the onset of seizure activity or 40 min. after the onset of seizure activity that was detected in the electroencephalographic record. When given immediately after seizure onset, the anticonvulsant ED<sub>50</sub> of intramuscular midazolam was significantly lower than that of intranasal midazolam, which in turn was significantly lower than sublingual midazolam at that time. At the 40-min. treatment delay, the anticonvulsant ED<sub>50</sub>s of intramuscular or intranasal midazolam did not differ and both were significantly lower than the sublingual route. Higher doses of midazolam were required to stop seizures at the 40-min. treatment delay time compared to immediate treatment. The speed of seizure control for intramuscular or intranasal midazolam was the same while sublingual midazolam acted significantly slower. Midazolam was effective in treating soman-induced seizures when given by all three routes, but with differences in potency and speed of action.

Nerve agent-induced seizures result from over-stimulation of susceptible brain circuits by abnormally high levels of the excitatory neurotransmitter acetylcholine that rapidly builds up after inhibition of the enzyme acetylcholinesterase by nerve agent [1]. These seizures, unless quickly stopped pharmacologically, rapidly progress to status epilepticus, a state of continuous seizure activity or episodes of seizure activity for greater than 30 min. with no recovery of consciousness between episodes. Status epilepticus itself is considered a medical emergency, and the longer seizures persist, the more difficult they are to stop pharmacologically [2]. Status epilepticus clinically responds (n.b. termination of seizures) only to a subset of anticonvulsant or antiepileptic

drugs. Benzodiazepines are typically the most effective class of compounds and are used as the first drug of choice in treatment of this condition. Nerve agent-induced status epilepticus also responds to benzodiazepines [3,4], as well as to anticholinergic compounds [5] that have no therapeutic benefit in the treatment of epilepsy or seizures induced by clinical states other than nerve agents.

The benzodiazepine diazepam is currently available in auto-injectors as an immediate field treatment to counteract nerve agent-induced seizures. However, diazepam is not very potent relative to other benzodiazepines for control of nerve agent seizures [3] and has slow bioavailability when given intramuscularly [6], the currently approved route of drug administration for an immediate field treatment of nerve agent casualties by military personnel. The maximum allowable dose of diazepam that can be administered in the field to a nerve agent casualty (30 mg; 0.43 mg/kg for a 70-kg human) was not able to reliably stop seizures elicited by the nerve agent soman in rhesus monkeys (~25% success rate). In contrast, the benzodiazepine midazolam (0.28–0.30 mg/kg) was highly successful (>90% rate) in terminating soman-induced seizures and time for seizure termination was twice as fast as with diazepam under similar conditions [7,8]. These same basic findings have also been demonstrated in extensive tests in a guinea pig model of nerve agent-induced seizures [4,6,9–11]. Midazolam has proved to be approximately twice as potent and controls seizures twice as fast as diazepam when the drugs are administered intramuscularly across tests of seizures elicited by six nerve agents. For these

Author for correspondence: John H. McDonough, US Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21040-5400, USA (fax 410 436-8377, e-mail john.mcdonough1@us.army.mil).

\*The opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army or the Department of Defense.

The experimental protocol was approved by the Animal Care and Use Committee of the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles in *the Guide for the Care and Use of Laboratory Animals* (National Research Council Publication No. 85–23, 1996) and the Animal Welfare Act of 1966 (P.L. 89–544), as amended, in an AAALAC, International accredited facility. This work was jointly supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division, and by a Cooperative Research and Development Agreement with Intranasal Therapeutics Inc., Lexington, KY, USA.

reasons, midazolam is being considered by the US military as a replacement anticonvulsant for diazepam for the treatment of nerve agent-induced seizures.

There are increasing numbers of clinical reports about the effectiveness of midazolam as an anticonvulsant for status epilepticus seizures when it is delivered either by the intranasal or buccal or sublingual route [12–24]. These routes of administration have been of special interest for effectively treating seizures in high-risk patients in a pre-hospital setting by parents or other caregivers with limited first aid skills. Intranasal or sublingual administration of anticonvulsants affords a rapid and simple way for immediate treatment of this potentially life-threatening condition and may be beneficial for treating nerve agent victims in a mass casualty situation. In this respect, Gilat *et al.* [20,21] reported that 1.0 or 1.5 mg/kg midazolam given intranasally was as effective as the same dose given intramuscularly in controlling seizures elicited by the nerve agent sarin in a guinea pig model. The present study was performed to more systematically compare the anticonvulsant effectiveness of midazolam to terminate nerve agent-induced seizures when it was administered by the intramuscular, intranasal or sublingual route.

## Materials and Methods

**Subjects.** Male Hartley guinea pigs (CrI:(HA) BR COBS; Charles River Laboratories, Kingston, NY, USA), weighing 250–300 g before surgery, served as subjects. They were individually housed in temperature ( $21 \pm 2^\circ$ ) and humidity ( $50 \pm 10\%$ ) controlled quarters that were maintained on a 12-hr light:dark cycle (lights on at 06:00) and received food and water *ad libitum* except during the experimental period.

**Surgery.** Approximately 1 week before experimentation, the animals were implanted with stainless-steel cortical screw electrodes to record electroencephalographic (EEG) signals. The animals were anaesthetized with isoflurane (3% induction, 1.5–2% maintenance; with oxygen) and set in a stereotaxic frame. Three cortical stainless-steel screw electrodes were implanted in the skull: two were placed bilaterally ~3.0 mm lateral from the midline and equidistant between bregma and lambda; the third was placed on the posterior calvarium as the reference electrode. Stainless-steel wires attached the screws to a miniature connector plug. The electrodes, wires and plug were encased in cranioplastic cement. The incision was sutured; the animal was removed from the frame, given the analgesic buprenorphine HCl (0.03 mg/kg, subcutaneously) and placed on a warming pad for at least 30 min. before being returned to the animal quarters.

**Materials.** Saline (0.9% NaCl) injection, United States Pharmacopeia (USP) and sterile water for injection were purchased from Cutter Laboratories Inc. (Berkeley, CA, USA). Pyridostigmine bromide was obtained from Hoffmann-La Roche Inc. (Nutley, NJ, USA) and pyridine-2-aldoxime methyl chloride (2-PAM) was purchased from Ayerst Laboratories Inc. (New York, NY, USA). Atropine sulfate was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Attane<sup>TM</sup> (isoflurane, USP) was purchased from Minrad Inc. (Bethlehem, PA, USA). Buprenorphine HCl was purchased from Reckitt Benckiser Pharmaceuticals Inc. (Richmond, VA, USA). The nerve agent soman was obtained from the US Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD, USA). The midazolam used for the intramuscular injections (Midazolam HCl Injection; 5.0 mg/ml) was purchased

Table 1.

Doses, concentrations and maximum volumes of midazolam used for the intranasal and oral routes of administration.

Dose (mg/kg)	Concentration (mg/ml)	Maximum volume (ml) <sup>1</sup>
0.56, 1.00	5.0	0.06
1.80	10.0	0.06
3.2, 5.6	20.0	0.09
10.0, <sup>2</sup> 18.0, <sup>3</sup> 32.0 <sup>3</sup>	50.0	0.09, <sup>2</sup> 0.21 <sup>3</sup>

<sup>1</sup>Volumes were calculated based on a 0.325-kg guinea pig.

<sup>2</sup>Dose used for both intranasal and sublingual route.

<sup>3</sup>Doses used for sublingual route only.

from Abbott Laboratories (Chicago, IL, USA; Lot 30-231-DK). The midazolam used for the intranasal and oral administrations was obtained from Intranasal Therapeutics Inc. (Lexington, KY, USA), under a cooperative research and development agreement, and was received as PN7007 Midazolam, 50 mg/ml, Lot no. ND0702 MZ. Also received from Intranasal Therapeutics Inc. was the diluent as PN7008 Midazolam Placebo, Lot no. ND0701 MZ. The midazolam and midazolam placebo solutions received from Intranasal Therapeutics Inc. were maintained under refrigeration in their original containers except during dilution procedures.

Pyridostigmine bromide was prepared in sterile water to a concentration of 0.052 mg/ml. Atropine sulfate (4 mg/ml) and 2-PAM (50 mg/ml) were prepared in saline as an admixture and administered 0.5 ml/kg. Soman was prepared in ice-cold saline to a concentration of 112 µg/ml. The midazolam used for the intramuscular injections (5 mg/ml) was used without dilution. For the intranasal and sublingual administration routes, different concentrations of midazolam were prepared from the midazolam and midazolam placebo diluent provided by Intranasal Therapeutics Inc. in an effort to equate, as best possible, the volume of midazolam administered across dose groups. These concentrations and maximum volumes are provided in table 1.

**Procedures.** Animals were typically tested in squads of eight on a given study day. On the day of study, the animals were randomly assigned to a route of administration and dose group until all groups were filled. The animals were weighed, placed in a recording chamber and at least 15 min. of baseline EEG recorded. EEGs were recorded using CDE 1902 amplifiers and displayed on a computer running Spike2 software (Cambridge Electronic Design Ltd., Cambridge, UK). The animals were then administered 0.026 mg/kg pyridostigmine intramuscularly, a dose determined to produce ~30% whole blood cholinesterase inhibition [25]. This dose of pyridostigmine never produced any abnormal behaviour in the animals, nor changed the appearance or waveform characteristics of the EEG as measured by power spectral analysis prior to administration of soman. Thirty minutes after pyridostigmine, the animals were challenged with soman 56 µg/kg, subcutaneously, and, 1 min. later, were treated intramuscularly with the admixed 2.0 mg/kg atropine and 25.0 mg/kg 2-PAM. They were then monitored for seizure onset by a technician and investigator well experienced with the appearance of nerve agent-induced EEG seizure activity in guinea pigs. Seizure onset required consensus agreement between the technician and investigator and was operationally defined as the appearance of  $\geq 10$  sec. of rhythmic high-amplitude spikes or sharp waves that were at least twice the baseline amplitude. Either immediately following seizure onset or 40 min. later, the animals were administered midazolam treatment at the pre-determined dose and route of administration. The immediate treatment study was performed first, followed by the 40-min. delay study. For intramuscular midazolam, the injection was given in the rear thigh muscle. For the intranasal and sublingual routes, a small piece of polyethylene tubing (PE-50) was placed over the end of a 23-gauge blunt stubbed needle that was fitted to a 1-ml syringe; approximately

1–2 mm of the tubing projected beyond the end of the needle. The tubing and needle were inserted approximately 4–5 mm up the animal's nares (intranasal route) or under the tongue (sublingual route), and half the volume of each dose was given on each side. Each dose X route X treatment time group consisted of six animals with the exception of 5.6 mg/kg, intramuscularly, 40-min. treatment group where  $n = 7$ . The EEG was continuously monitored for at least 4 hr after treatment and for 30 min. at 24 hr after exposure. Each animal was rated as having the seizure terminated or not terminated based on the overall appearance of the EEG record at the end of the experimental day and during the 24-hr recording. Evaluation and categorization of the EEG response by an individual animal to treatment was performed by a technician and investigator both well experienced with the appearance of nerve agent-induced EEG seizure activity. The overall rating and timing of different events required consensus agreement between both individuals who were aware of the treatment conditions of an individual animal. To be rated as having the seizure terminated, all spiking and/or rhythmic waves had to stop and the EEG remain normal at all subsequent observation times. For each animal in which the seizure was terminated, the latency to seizure termination was measured as the time from when the animal received midazolam treatment to the last observable epileptiform event in the EEG. These evaluation procedures and operational criteria for seizure control are identical to those used in previous studies utilizing this animal model [3,5,6,9–11].

**Data analysis.** Dose-effect curves for seizure termination were developed using quantal response probit analysis for each route of administration at each treatment time using 3–5 drug doses. The data were analysed using the SAS/STAT (Statistical Analysis Software, Cary, NC, USA) probit programme. Additionally, a SAS programme was used to compare the relative mean potencies of administration routes and times at the 50% response dose along with their respective 95% confidence intervals [26]. These relative mean potencies are the ratio of the 50% response doses for two routes for a given time or two times for a given route. With this

determination, if the 95% confidence interval of the resultant ratio included the value of 1, then the  $ED_{50}$ s for the two routes (or times) were considered similar; if the 95% confidence interval excluded the value of 1, then the  $ED_{50}$ s for the routes (or times) were significantly different. Seizure termination latencies were evaluated by the Kruskal-Wallis one-way ANOVA on ranks followed by Dunn's multiple comparison procedure using GraphPad Prism Software, version 4.03 (GraphPad Software Inc., La Jolla, CA, USA).

## Results

Midazolam proved to be an effective anticonvulsant against seizures elicited by the nerve agent soman when given immediately after seizure onset or when treatment was delayed for 40 min. after seizure onset. Figure 1 displays an example of that anticonvulsant effect. There was a clear dose dependency for this anticonvulsant effect by each route of administration. Additionally, there were significant differences in drug potency linked to the different routes of administration.

### Dose-effect relationships.

Tables 2 and 3 display the response of the different animals treated at seizure onset or 40 min. after seizure onset, respectively, with the varying doses of midazolam by the different routes of administration. As can be seen, there were increasing numbers of animals within a group displaying an anticonvulsant response as the treatment dose of midazolam was increased, regardless of route of administration and time of treatment. With the sublingual route of treatment at the 40-min. delay after seizure onset, it was not possible to achieve

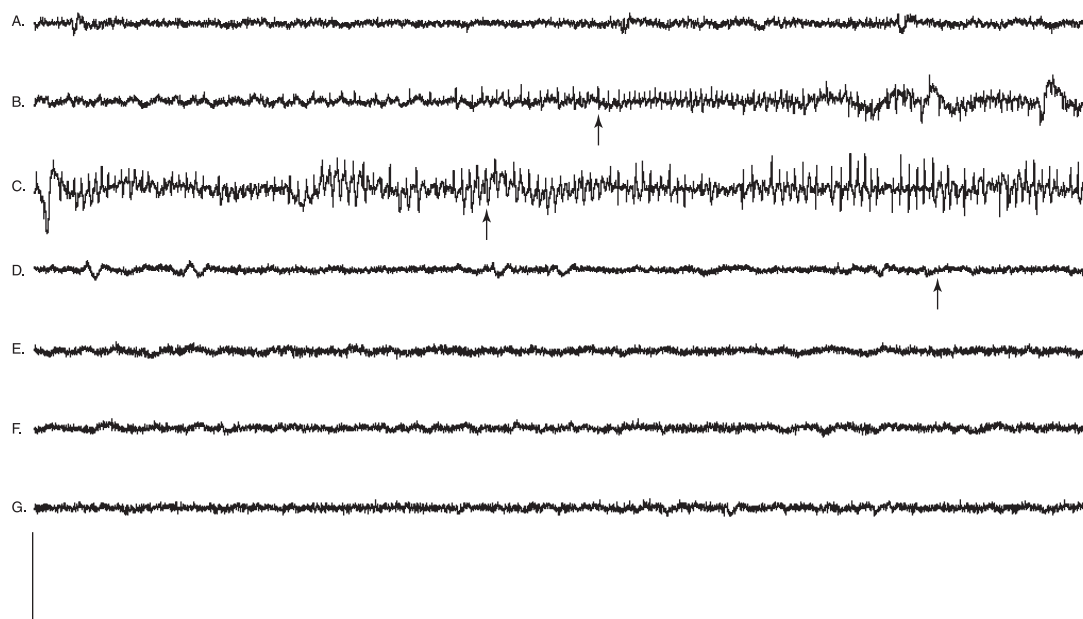


Fig. 1. Example of midazolam control of soman-induced seizure activity. Animal received 1.00 mg/kg midazolam, intranasal, immediately after seizure onset. (A) baseline EEG activity prior to soman exposure; (B) onset of soman-induced seizure (arrow), note the repetitive spiking; (C) EEG activity at the time intranasal midazolam was administered (arrow); (D) termination of seizure activity 3 min. 57 sec. after intranasal administration of midazolam (arrow), spike activity stopped just prior to this strip, and the repeated slow waves were considered to also represent residual epileptiform activity; normalized EEG activity at all times subsequent to termination (E) 1 hr after midazolam; (F) 4 hr after midazolam; (G) record next morning, ~24 hr after midazolam. Calibration: 0.5 mV, 5 sec.

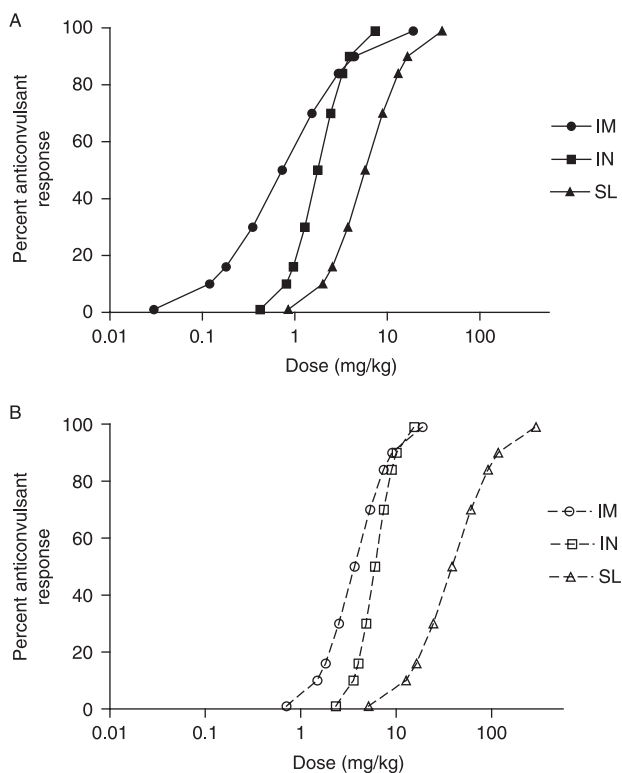


Fig. 2. Fitted dose-effect curves calculated from the results of the probit analysis for anticonvulsant effects of midazolam administered by three different routes. Panel A – immediate treatment after onset of soman-induced seizures; Panel B – treatment 40 min. after onset of soman-induced seizures.

a 100% anticonvulsant response at the highest dose tested, in this case, 32.0 mg/kg. It was determined that testing the next higher dose (56.0 mg/kg) was impractical due to limitations in drug concentration (a dose of 56.0 mg/kg would require volumes in excess of 0.3 ml) and the fact that such a dose is almost a log unit greater than the sublingual immediate treatment  $ED_{50}$  dose of 5.77 mg/kg.

The anticonvulsant  $ED_{50}$ s and confidence limits of midazolam for the different route administration at the two different treatment times are given in table 4. The fitted dose-effect curves determined from these results are displayed in fig. 2. When midazolam was given immediately after seizure onset, the  $ED_{50}$  (0.73 mg/kg) for the intramuscular route was significantly more potent than that for the intranasal (1.77 mg/kg) route, and both of these  $ED_{50}$ s were significantly more potent than the  $ED_{50}$  for the sublingual (5.77 mg/kg) route. At the 40-min. treatment delay, the  $ED_{50}$ s for the intramuscular (3.67 mg/kg) and intranasal (6.01 mg/kg) routes of administration were not statistically different, while the  $ED_{50}$  for the sublingual route (38.69 mg/kg) was significantly higher than for either of the other two routes. When compared across treatment times, the  $ED_{50}$ s for each route of administration were significantly lower at the seizure onset treatment condition than were their respective  $ED_{50}$ s at the 40-min. treatment delay condition.

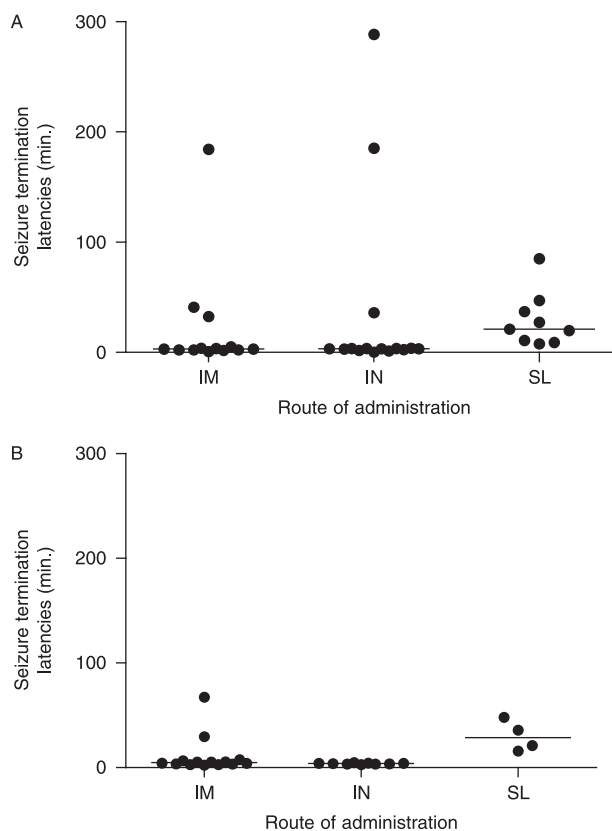


Fig. 3. Scatter plots of the latencies for seizure termination as a function of the route of midazolam administration. These data were collapsed across doses at each treatment time; points represent data for individual animals, horizontal lines are medians. Panel A is the treatment immediately after seizure onset condition; the sublingual route produced significantly elevated ( $P < 0.05$ ) latencies compared to either the intramuscular or intranasal route, which did not differ from one another. Panel B is the treatment 40 min. after seizure onset condition; the sublingual route produced significantly elevated ( $P < 0.01$ ) latencies compared to the intranasal route; there was no difference between the intramuscular versus intranasal or intramuscular versus sublingual routes.

#### Seizure termination latencies.

All seizure termination latencies for each route of administration, regardless of dose, were collapsed, and comparisons between the different routes were evaluated by Kruskal-Wallis one-way ANOVAS on ranks for each treatment time. These data are displayed in fig. 3. The analyses showed significant differences between the median times for seizure termination by midazolam among the different routes of administration at both the seizure onset treatment condition ( $H = 8.05$ ,  $df = 2$ ,  $P = 0.02$ ) and the 40-min. treatment delay condition ( $H = 9.04$ ,  $df = 2$ ,  $P = 0.01$ ). These differences were further evaluated using the Dunn's multiple comparison procedure. With the seizure onset treatment condition, the seizure termination latencies of the sublingually treated animals were significantly longer than those of animals treated by the intramuscular or intranasal routes, while the latencies of the intramuscular and intranasal routes were not statistically

Table 2.

Summary of anticonvulsant effects of midazolam administered by the intramuscular (IM), intranasal (IN) or sublingual (SL) route in individual test animals treated at the onset of soman-induced seizures.

IM	0.18 mg/kg	0.32 mg/kg	0.56 mg/kg	1.00 mg/kg	1.80 mg/kg
	X (3:04:19)	X (0:02:34)	X (0:41:11)	X (0:02:28)	X (0:00:48)
	O	O	O	X (0:05:14)	X (0:03:58)
	X/O [0:01:15] >4 hr	O	O	O	X (0:03:14)
	O	O	O	X (0:03:30)	X (0:01:53)
	O	O	X/O [0:04:51] 2:43:45	O	X (0:03:05)
	X (0:02:26)	O	O	X (0:32:37)	O
IN	0.56 mg/kg	1.00 mg/kg	1.80 mg/kg	3.20 mg/kg	5.60 mg/kg
	O	O	O	X (0:03:42)	X (0:00:15)
	O	X (0:03:57)	O	X (4:48:33)	X (0:03:30)
	O	O	X (0:02:33)	X (0:03:29)	X (0:03:30)
	O	X (0:01:50)	O	O	X (0:03:16)
	O	O	X (3:05:28)	X (0:03:29)	X (0:03:40)
	O	O	O	X (0:01:24)	X (0:36:11)
SL	1.00 mg/kg	1.80 mg/kg	3.20 mg/kg	5.60 mg/kg	10.00 mg/kg
	O	O	O	O	X (0:09:00)
	O	O	O	X/O [0:11:46] >4 hr	X (0:07:45)
	O	O	O	X (0:47:05)	X (1:25:02)
	X (0:37:05)	O	O	O	X (0:10:47)
	O	O	O	O	X (0:21:04)
	O	O	X/O [2:00:24] >4 hr	X (0:27:20)	X (0:19:49)

O = Indicates an animal in which seizure was not terminated by treatment.

X = Indicates an animal in which seizure was terminated by treatment; latency (h:mm:ss) to seizure termination within parenthesis.

X/O = Indicates an animal in which there was an initial seizure termination, but seizure returned either same day or on recording 24 hr later; animal considered a treatment failure; latency [h:mm:ss] to initial seizure control within brackets, time seizure noted to return in next column.

Table 3.

Summary of anticonvulsant effects of midazolam administered by the intramuscular (IM), intranasal (IN) or sublingual (SL) route in individual test animals treated 40 min. after the onset of soman-induced seizures.

IM	1.80 mg/kg	3.20 mg/kg	5.60 mg/kg	10.00 mg/kg
	X/O [0:04:05] >4 hr	X/O [0:05:55] >4 hr	X (0:29:35)	X (0:06:43)
	X/O [0:36:52] 3:38:42	X/O [0:40:17] >4 hr	X (0:05:42)	X (0:02:23)
	X/O [0:10:29] 2:34:13	X (0:05:25)	O	X (0:03:36)
	X (1:07:17)	X (0:05:20)	X (0:07:41)	X (0:03:20)
	X/O [0:10:08] >4 hr	X (0:02:51)	X/O [0:04:39] >4 hr	X (0:04:14)
	X/O [0:15:04] 3:05:10	X/O [0:59:14] 1:54:10	X (0:04:18)	X (0:02:58)
			X/O [0:03:46] >4 hr	
IN	1.80 mg/kg	3.20 mg/kg	5.60 mg/kg	10.00 mg/kg
	X/O [0:10:32] >4 hr	X/O [0:13:03] 2:13:39	X/O [0:08:43] 2:50:27	X (0:03:26)
	X/O [0:04:01] 2:49:40	X/O [0:08:56] 3:01:19	O	X (0:04:28)
	O	O	X/O [0:16:26] 3:00:30	X (0:04:03)
	X/O [0:15:50] >4 hr	X/O [0:04:35] >4 hr	X (0:03:29)	X (0:04:03)
	O	X (0:02:50)	X/O [0:10:24] >4 hr	X (0:03:53)
	O	X/O [0:10:21] >4 hr	X/O [0:09:05] 3:26:39	X (0:03:30)
SL	10.00 mg/kg	18.00 mg/kg	32.00 mg/kg	
	O	X (0:48:08)	X/O [0:42:08] >4 hr	
	X/O [0:10:36] >4 hr	X/O [1:34:59] 2:23:32	X/O [0:05:21] >4 hr	
	X/O [0:43:04] 3:19:10	X/O [0:55:51] 1:04:53	X (0:15:45)	
	O	X/O [0:37:32] 1:06:53	X/O [1:06:34] 1:17:20	
	X/O [0:04:10] >4 hr	X (0:21:02)	X (0:35:58)	
	X/O [1:13:53] 1:14:53	X/O [0:42:23] >4 hr	X/O [1:08:09] 2:07:03	

O = Indicates an animal in which seizure was not terminated by treatment.

X = Indicates an animal in which seizure was terminated by treatment; latency (h:mm:ss) to seizure termination within parenthesis.

X/O = Indicates an animal in which there was an initial seizure termination, but seizure returned either same day or on recording 24 hr later; animal considered a treatment failure; latency [h:mm:ss] to initial seizure control within brackets, time seizure noted to return in next column.

different. At the 40-min. treatment delay condition, the only significant group difference was that the seizure termination latencies for the sublingual route of administration were longer than those for the intranasal route.

#### Seizure recurrence.

One finding of this study was the large numbers of animals in which seizures returned following an initial anticonvulsant effect. These animals were considered as treatment failures,

Table 4.

ED<sub>50</sub>s and 95% confidence limits for the anticonvulsant dose-effect curves for midazolam given by the different routes of administration at the two treatment times after soman seizure onset.

Route	Treatment time after seizure onset	ED <sub>50</sub> (mg/kg)	95% confidence limits (mg/kg)
Intramuscular	Immediate	0.73	0.37–1.47
	40 min.	3.67	2.45–5.49
Intranasal	Immediate	1.77	1.24–2.54
	40 min.	6.01	4.49–8.04
Sublingual	Immediate	5.77	3.54–9.41
	40 min.	38.69	13.94–107.42

because seizure activity was not permanently controlled by the drug treatment. Virtually, all animals in which this occurred had been treated with doses of midazolam less than the calculated ED<sub>50</sub> for the particular route of administration. This phenomenon occurred significantly more frequently in animals in the 40-min. treatment delay condition (33 of 68 animals) than in the seizure onset immediate treatment condition (4 of 90 animals) ( $\chi^2 = 39.55$ ,  $df = 1$ ,  $P < 0.001$ ) and appeared with approximately equal frequency across the different routes of treatment. An example of the EEG record of such an animal is shown in fig. 4. In these cases, seizure activity would terminate in the EEG

record in the same fashion as other successful treatments, but would return after some period of time, with approximately 50% of the cases occurring >4 hr after the initial seizure control. When the seizure activity returned, the initial EEG events typically were short 5- to 30-sec. rhythmic bursts of increased amplitude with a sharp dominant frequency of 7–9 Hz and with a secondary minor sharp peak at 23–25 Hz. These events tended to repeat themselves, becoming successively longer in duration, with greater amplitude, with peak frequencies shifting to 12–18 Hz and with shorter periods between events. Eventually, full electrographic seizures developed, most lasting 1–2 min., with significantly greater amplitudes and dominant frequencies of 2–6 Hz. These seizure episodes would then repeat at varying intervals for the rest of the recording period.

### Discussion

The results show that midazolam is capable of terminating seizures elicited by the nerve agent soman when administered by all three of the delivery routes that were tested. However, there were significant differences between the anticonvulsant potency of midazolam and its speed of action that were linked to both the different routes of administration and the time of dosing after seizure onset. In addition, it was noted

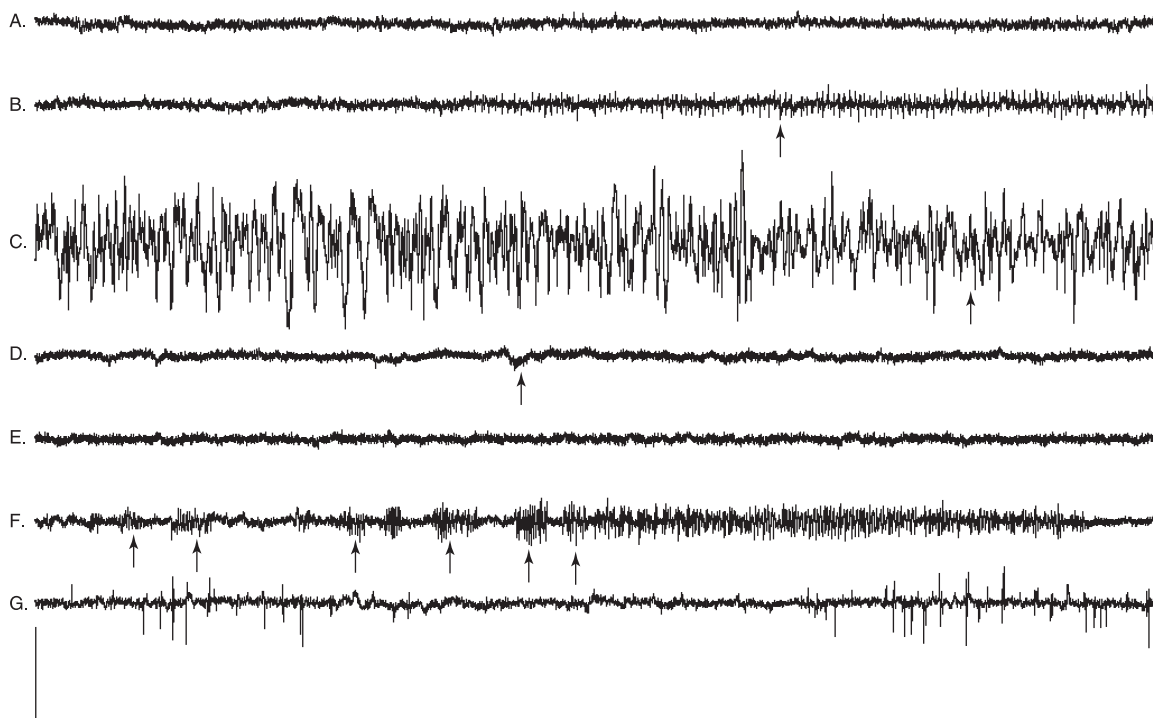


Fig. 4. Example of an initial control of seizure activity by midazolam and subsequent seizure recurrence. Animal received 5.6 mg/kg midazolam, intranasal, 40 min. after seizure onset. (A) baseline EEG activity prior to soman exposure; (B) onset of soman-induced seizure (arrow), note the repetitive spiking; (C) EEG activity 40 min. after seizure onset at the time intranasal midazolam was administered (arrow); (D) termination of seizure activity 16 min. 26 sec. after intranasal administration of midazolam (arrow), spike activity stopped prior to this strip, and the repeated slow waves were considered to also represent residual epileptiform activity; (E) EEG activity remains normal 1 hr after seizure termination; (F) seizure activity returns 3 hr and 30 sec. after seizure termination with repetitive short bursts of high amplitude 7–9 Hz waves (arrows) that culminated in a prolonged discharge; there were six additional episodes like this over the next hour of recording; (G) record next morning, ~24 hr after midazolam; note continued spiking activity. Calibration: 0.5 mV, 5 sec.

that there was a high frequency of seizure recurrence following an initial anticonvulsant effect that was associated with delayed treatment with less than optimal therapeutic doses.

For all practical purposes, midazolam given by the intramuscular or intranasal routes produced equivalent anticonvulsant effects. Although the intramuscular route resulted in a significantly lower  $ED_{50}$  than the intranasal route at the seizure onset immediate treatment time, the speed of anticonvulsant effect was equivalent for the two routes. At the 40-min. treatment delay time, there were no differences between the intramuscular and intranasal routes in either  $ED_{50}$ s or speed of anticonvulsant effect. The fact that there were no differences in the speed of seizure control between the intramuscular and intranasal routes at either treatment time would seem to indicate that the anticonvulsant pharmacodynamics of midazolam were essentially equivalent by these two routes. It should be noted that the intramuscular  $ED_{50}$ s and seizure control latencies for midazolam obtained in this study are virtually identical to those obtained in previous studies using this model [3,11] and are also in close agreement with the results of Gilat *et al.* [20].

The sublingual route of administration for midazolam was also able to produce reliable anticonvulsant effects, but significantly higher doses were required and the speed of the effect was slower than with either of the other routes. A less efficient absorption of the dose from the sublingual tissue than from an intramuscular injection or through the nares could account for the need for a higher dose and the slower anticonvulsant effect. Because drug pharmacokinetics were not incorporated into this study, it can only be stated what doses were delivered and not necessarily absorbed. While it could be argued that the sublingual administration used in this study was only an approximation of human sublingual or buccal drug administration, the results still indicate a slower pharmacodynamic effect than with either the intramuscular or intranasal route, presumably due to less efficient or rapid drug absorption. It is interesting to note that the increase in  $ED_{50}$ s between the seizure onset immediate treatment condition and 40-min. treatment delay condition was roughly of the same magnitude regardless of the route of administration. This is interpreted to indicate that the increase in  $ED_{50}$  is due to some common factor of the seizure condition that is a function of seizure duration, because it was independent of the route of drug administration.

The phenomenon of seizure recurrence following an initial anticonvulsant effect is something that had been occasionally observed before in other of our studies of benzodiazepine treatment of nerve agent-induced seizures [3,5]. The findings from this study show that a long delay from seizure onset until treatment and less than optimal therapeutic doses were the two factors associated with seizure recurrence. There were significantly greater numbers of animals in which seizures returned in the 40-min. treatment group and, virtually, all the animals had been treated with doses less than the  $ED_{50}$ . Route of drug administration was not a contributing factor. There was no notable feature in the EEG or in the behaviour of the animals that would

predict that a seizure would recur. In the animals in which seizures recurred, the initial seizure termination latencies were no different, neither shorter nor longer, than in the animals in which the seizure stayed off. It should be noted that seizures stayed off for a minimum of 65 min. and more commonly for >2 hr before the first EEG sign that they were to recur in a given animal. In a practical sense, this would be sufficient time for a casualty to be transported from the site of exposure to a definitive treatment facility. Whether a repeated midazolam treatment at the first EEG evidence that the seizure was to recur would prevent this seizure recurrence is a question for further research.

While the results of this study show that intranasally and sublingually administered midazolam are fully capable of controlling seizures elicited by the nerve agent soman either temporarily or permanently, there is a question of whether either of these routes would be practical in treating a casualty exposed to nerve agent vapour. Inhalation exposure to nerve agents produces copious oral and nasal secretion. Sidell [27] has described cases of inhalation exposure where 'noses ran like a drippy faucet', and that especially severe exposure cases required repeated oral-nasal suction to assist respiration. Whether intranasal or sublingual midazolam administration would be as effective under such conditions is somewhat problematic. Because the anticonvulsant would also be administered with the standard nerve agent antidote atropine, which by itself is capable of reducing such secretions, this may not be as great a concern. In addition, researchers have not noted a reduced effectiveness of intranasally or sublingually/buccally administered midazolam in controlling seizures in clinical tests due to the interference of oral-nasal secretions. Likewise, increased nasal secretions from rhinitis have been shown to not significantly affect the bioavailability of other compounds delivered by the intranasal route [28,29].

In summary, the present study compared the ability of midazolam when given by different routes of administration to terminate seizures produced by the nerve agent soman. The speed of anticonvulsant effectiveness of midazolam was the same for the intramuscular and intranasal routes of administration at either the immediate or 40-min. delayed treatment times, while intramuscularly administered midazolam was more potent only at the immediate treatment time. Midazolam administered by the sublingual route required significantly higher doses and was slower in acting to produce an anticonvulsant effect than by either of the other two routes at both treatment times. The results show that intranasal and sublingual routes would be effective in treatment of nerve agent-induced seizures, and that intranasal midazolam administration would act clinically as rapidly as intramuscular administration.

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